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**Heparan/Chondroitin Sulfate Biosynthesis. Structure and Mechanism of Human Glucuronyltransferase I**

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**ABSTRACT:** Human beta1,3-glucuronyltransferase I (GlcAT-I) is a central enzyme in the initial steps of proteoglycan synthesis. GlcAT-I transfers a glucuronic acid moiety from the uridine diphosphate-glucuronic acid (UDP-GlcUA) to the common linkage region trisaccharide Gal beta 1-3Gal beta 1-4Xyl covalently bound to a Ser residue at the glycosaminylglycan attachment site of proteoglycans. We have now determined the crystal structure of GlcAT-1 at 2.3 Å in the presence of the donor substrate product UDP, the catalytic Mn(2+) ion, and the acceptor substrate analog Gal beta 1-3Gal beta 1-4Xyl. The enzyme is a alpha/beta protein with two subdomains that constitute the donor and acceptor substrate binding site. The active site residues lie in a cleft extending across both subdomains in which the trisaccharide molecule is oriented perpendicular to the UDP. Residues Glu(227), Asp(252), and Glu(281) dictate the binding orientation of the terminal Gal-2 moiety. Residue Glu(281) is in position to function as a catalytic base by deprotonating the incoming 3-hydroxyl group of the acceptor. The conserved DXD motif (Asp(194), Asp(195), Asp(196)) has direct interaction with the ribose of the UDP molecule as well as with the Mn(2+) ion. The key residues involved in substrate binding and catalysis are conserved in the glucuronyltransferase family as well as other glycosyltransferases.